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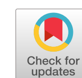
Research paper

Soluble fibre supplementation with and without a probiotic in adults with asthma: A 7-day randomised, double blind, three way cross-over trial



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SUMMARY

Background: Soluble fibre modulates airway inflammation in animal models. The aim of this study was to investigate the effects of soluble fibre supplementation, with and without a probiotic, on plasma short chain fatty acids (SCFA), airway inflammation, asthma control and gut microbiome in adults with asthma.
Methods: A randomised, double-blinded, placebo controlled 3-way cross-over trial in 17 subjects with stable asthma at the Hunter Medical Research Institute, Newcastle, Australia. Subjects received 3×7 day oral interventions in random order; soluble fibre (inulin 12 g/day), soluble fibre + probiotic (inulin 12 g/day + multi-strain probiotic >25 billion CFU) and placebo. Plasma SCFA, sputum cell counts and inflammatory gene expression, asthma control gut microbiota, adverse events including gastrointestinal symptoms were measured.
Findings: There was no difference in change in total plasma SCFA levels (μmol/L) in the placebo versus soluble fibre (Δmedian [95% CI] 16·3 [−16·9, 49·5], $p = 0{\cdot}335$) or soluble fibre+probiotic (18·7 [−14·5, 51·9], $p = 0{\cdot}325$) group. Following the soluble fibre intervention there was an improvement in the asthma control questionnaire (ACQ6) (Δmedian (IQR) -0·35 (−0·5, −0·13), $p = 0{\cdot}006$), sputum %eosinophils decreased (−1.0 (−2·5, 0), p = 0·006) and sputum histone deacetylase 9 (HDAC9) gene expression decreased (−0.49 (−0.83, −0.27) $2^{-\Delta Ct}$, $p = .008$). Individual bacterial operational taxonomic units changed following both inulin and inulin+probiotic arms.
Interpretation: Soluble fibre supplementation for 7 days in adults with asthma did not change SCFA levels. Within group analysis showed improvements in airway inflammation, asthma control and gut microbiome composition following inulin supplementation and these changes warrant further investigation, in order to evaluate the potential of soluble fibre as a non-pharmacological addition to asthma management.
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1. Introduction

Asthma is a chronic inflammatory airways disease, affecting >10% of adults in many westernised countries [1]. There is increasing evidence that a western style diet, which is typically low in fibre, is associated with increased risk of asthma. In humans, dietary fibre intake has been linked with reduced systemic inflammation in both healthy and diseased adults [2,3]. In asthma, both evidence from animal models and human observational data has shown that dietary fibre intake is also relevant in the lungs, providing a protective effect against eosinophilic airway inflammation [4,5]. Furthermore, using a single dose of soluble fibre (3.5 g inulin), we have shown a significant decrease in airway inflammation in adults with asthma [6]. Two other short term randomised controlled trials reported that soluble fibre (with and without probiotic) supplementation in asthma reduced systemic inflammation and improved lung function [7] and airway hyperreponsiveness [8].

The anti-inflammatory benefits of dietary fibre intake have largely been ascribed to the effects of fermentable soluble fibres. Soluble fibres resist digestion in the small intestine and are fermented by bacteria in

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Research in context

Evidence before this study

Soluble fibres are fermented by bacteria in the colon, giving rise to biologically active by-products, including the short chain fatty acids (SCFA). Soluble fibres also act as prebiotics, leading to an expansion of SCFA-producing taxa, further enhancing SCFA biosynthesis. Studies in animal models show that SCFAs have anti-inflammatory effects in immune cells via mechanisms such as G protein coupled receptor (GPR) 41/43 activation and suppression of histone deacetylases (HDACs). In a systematic review and meta-analyses we have shown that soluble fibre supplementation decreases systemic inflammation, including CRP, IL6, and TNFα. We conducted the first in-human study to show a reduction in airway inflammation in asthma following soluble fibre intervention. Asthmatic adults (n = 29) consumed a single dose of soluble fibre (3·5 g inulin) or control. At 4 h after inulin supplementation, GPR41 & GPR43 gene expression were upregulated in sputum cells and sputum neutrophils, macrophages, IL-8 and exhaled nitric oxide decreased. These promising results have led to the development of the longer term, 7 day intervention, described in this manuscript. Two other randomised controlled trials reported that short term soluble fibre (with and without probiotic) supplementation in asthma reduced systemic inflammation and improved lung function and airway hyperreponsiveness respectively.

Added value of this study

We have completed a 7 day trial examining the effects of soluble fibre (12 g/day inulin) on airway inflammation, gut microbiome composition and clinical markers of asthma. Asthmatic adults (n = 17) consumed soluble fibre (inulin, 12 g/day), soluble fibre + probiotic, or placebo for 7 days. Following soluble fibre supplementation, within group posthoc analysis demonstrated an improvement in asthma control (ACQ6), a reduction in airway eosinophils, inhibition of HDAC9 expression in sputum cells and changes in individual bacterial operational taxonomic units in the gut compared to baseline. Importantly, following inulin supplementation, in the subset of subjects with poorly controlled asthma at baseline (ACQ6 > 0.75; n = 8), 100% of subjects had an improvement in asthma control, with 63% reaching the minimum clinically important difference (MCID, 0·5).

Implications of all of the evidence

Soluble fibre supplementation modulates the gut microbiome and posthoc analysis has identified anti-inflammatory actions in the airways, that could provide a non-pharmacological addition to asthma management, and require further investigation.

the colon, giving rise to biologically active by-products, including the short chain fatty acids (SCFA), acetate, butyrate and propionate. By providing a selective growth substrate, prebiotic fibres result in an expansion SCFA-producing taxa, such as *Bifidobacterium* and *Lactobacillus*, [9] and an increased capacity for SCFA biosynthesis. A temporary increase in SCFA-biosynthetic capacity can also be achieved through the use of probiotics, most commonly strains of *Bifidobacterium* and *Lactobacillus* [10,11]. Probiotics are typically delivered as either a supplement (tablet/powder) or via functional foods (e.g. yogurts and cultured drinks). Supplementation with a synbiotic (combination of prebiotic soluble fibre and probiotics) is hypothesised to have a greater effect on inflammation than prebiotics alone.

SCFAs have attracted significant interest in recent years due to their anti-inflammatory properties. Mechanisms proposed to be mediated by SCFA include activation of G protein-coupled receptor (GPR) 41 and GPR43 [12], also known as free fatty acid receptors (FFAR), and epigenetic regulation via inhibition of histone deacetylase (HDAC) enzyme activity [13]. Both mechanisms inhibit NFκB activity, leading to anti-inflammatory effects including: reduced neutrophil migration, reduced production of pro-inflammatory cytokines, reduced adhesion molecule expression and enhanced production of regulatory T cells (Treg) [14]. This highlights the potential for soluble fibre to provide a non-pharmacological addition to asthma management.

To our knowledge this is the first study in asthma to examine the effect of supplementation with the soluble fibre inulin. This study aimed to examine the effect of a prebiotic soluble fibre, with and without a probiotic, on plasma and faecal SCFA, airway inflammation, asthma control, lung function, gut microbiota and molecular mechanisms, in adults with asthma. Adverse events were monitored, including gastrointestinal symptoms assessed using the gastrointestinal symptom rating scale (GSRS).

2. Methods

2.1. Study design & population

The study was a double-blind, randomised, placebo-controlled three way cross-over trial of inulin, inulin+probiotic or placebo, for 7 days with a 2 week run-in and 2 week wash-out periods between treatments (Fig. 1). This study was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12615000368538), conducted at the Hunter Medical Research Institute (HMRI) Newcastle, Australia in accordance with the Declaration of Helsinki with approval from the Hunter New England Human Research Ethics Committee (15/03/18/3·03) and written informed consent was obtained. The full study protocol is available: http://hdl.handle.net/1959.13/1397040. Adults with doctor-diagnosed, stable asthma were recruited through HMRI volunteer databases and by advertisement. Exclusion criteria included: other respiratory conditions, current smoking (within 6 months), pregnancy or breastfeeding, diagnosed bowel or intestinal disorders, current use of nutritional, fibre or probiotic supplements (within 4 weeks), use of medications known to influence systemic inflammation.

2.2. Interventions

The inulin treatment consisted of 6 g of inulin twice daily (Frutafit® CLR, Sensus, The Netherlands) and a placebo capsule daily (microcrystalline cellulose). The inulin+probiotic treatment consisted of 6 g of inulin twice daily and a multi-strain probiotic capsule daily, (Caruso's Natural Health, Sydney, NSW, Australia) containing *Lactobacillus acidophilus* LA-5 (7·5 billion colony forming units (CFU)), *Lactobacillus rhamnosus* GG (8·75 billion CFU) and *Bifidobacterium animalis* subspecies *lactis* BB-12 (8·75 billion CFU) and coated with rate-controlling hypromellose polymer for sustained release. Survival of the probiotic strains in the GI tract using this delivery form is demonstrated in previous efficacy studies [15]. The placebo treatment consisted of 6 g of maltodextrin powder twice daily (Bulk Nutrients, Grove, Tasmania, Australia) and a placebo capsule. Inulin and placebo powders had similar appearance and taste with identical packaging. Subjects were instructed to mix powder sachets (inulin or placebo) with water and drink immediately morning and night, and to take the capsule in the morning only, both before food.

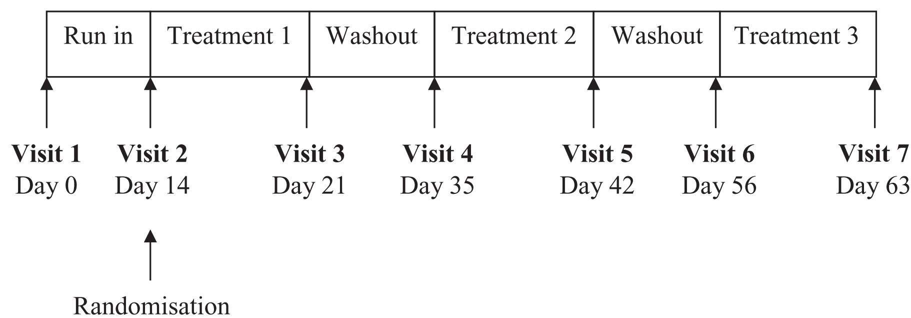

Fig. 1. Trial Schema. Following screening at visit 1, eligible subjects commenced a fibre-controlled background diet which was maintained for the duration of the study. After the initial 14 days on the fibre-controlled diet, subjects commenced a randomised cross-over supplementation trial, which included 3 treatment arms [inulin, inulin+probiotic or placebo]. Each treatment was used for 7 days, with a 14 day wash out period between each treatment. All outcomes were measured, and blood, sputum and faecal samples were collected at visits 2–7.

2.3. Randomisation, masking and data management

Treatment order was randomly allocated via unique computer-generated randomisation codes using a 3 × 3 latin square method by an independent statistician. Codes were concealed in an opaque envelope and accessed by investigators on study completion. Investigators and participants were blinded to the treatment. On enrolment, subjects were assigned a study code, which was used to collect and store all study data. Data was collected in hard copy and monitored, then re-identifiable data was entered into a secure, password protected, electronic database designed and used exclusively for the study. Hard copies of files will be archived in locked facilities for a period of 15 years.

2.4. Outcomes

The primary outcome of the study was plasma SCFA. Based on previous studies [16], we hypothesised that we would observe a change in plasma acetate of ~1SD (of the difference in response of matched pairs). To have 90% power to detect a difference between groups, with a two tailed type 1 error of 0·017 (for comparison of 3 groups), we required $n = 16$ subjects. Secondary outcomes were airway inflammation (sputum cell counts and FeNO), faecal SCFA and microbiota, lung function (forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC)), asthma control questionnaire 6 (ACQ6), gastrointestinal symptom rating scale (GSRS), blood and sputum cell HDAC1-11, GPR41 and GPR43 expression, total HDAC and histone acetyl transferase (HAT) activity. With 16 subjects, the study also had 90% power to detect a 1SD difference between groups in each of the secondary outcome measures, with a two tailed type 1 error of 0·017.

2.5. Procedures

Subjects were screened to confirm eligibility (visit 1), then instructed to consume a fibre controlled background diet for 2 weeks prior to the study commencing, and for the entire duration of the study. Subjects were provided with a written study diary to record daily treatment adherence and adverse events, defined as any untoward medical occurrence during the study intervention period. At each visit, gastrointestinal symptoms were also assessed using the Gastrointestinal Symptom Rating Scale (GSRS) [17,18]. Adherence to the intervention was monitored at each visit by 24-h food recall, pill/sachet countback and diary assessment. No changes to asthma medications were made during the study.

Subjects underwent assessment before and after each 7 day treatment arm, including exhaled nitric oxide fraction (FeNO) measurement according to the ATS/ERS criteria (Ecomedics CLD 88sp Analyser, Ecomedics, Duernten, Switzerland) [19], spirometry (MedGraphics, CPFS/D and BreezeSuite software, MedGraphics, Saint Paul, Minnesota, USA) [20], sputum induction coupled with bronchial provocation using nebulised (ULTRA-NEB™ ultrasonic nebuliser, DeVilbiss, Model 2000, Tipton, West Midlands, United Kingdom) hypertonic (4·5%) saline) [20] and asthma control (ACQ6) [18]. Predicted lung function was calculated using NHANES III reference values, including %predicted FEV_1 and %predicted FVC [21]. Fasting (≥12 h) blood samples were collected and subjects provided a stool sample (collected within 12 h) which was passed into a collection tray, transferred to a sterile specimen jar, then frozen immediately and stored at −20 °C until analysis.

Sputum collected by induction during hypertonic saline challenge was processed and analysed as previously described [22]. Airway responsiveness was assessed using dose response slope (DRS), calculated as %fall in FEV_1 per mL saline and provocation dose resulting in 15% fall in baseline FEV_1 (PD15). Sputum cell counts of eosinophils were used to classify subjects by asthma inflammatory phenotype, with non-eosinophilic asthma defined by sputum eosinophils <3% and eosinophilic asthma defined by sputum eosinophils ≥3% [22]. RNA was extracted from mucus plugs and converted to cDNA as previously described [23]. For gene expression analysis, Taqman qPCR primer and probes for HDAC subtypes (1−11), GPR43 and GPR41 were combined with cDNA and Taqman gene expression master mix as per manufacturer's instructions in duplicate real-time PCR reactions (7500 Real Time PCR System: Applied Biosystems). Analysis was performed on the change in cycle threshold (ΔCt) between the target gene compared with the housekeeping gene 18S rRNA, calculated using $2^{-\Delta Ct}$ (×10 [6]) relative to 18S [23].

Peripheral blood mononuclear cells were isolated from whole blood using the Ficoll-Paque PLUS (GE healthcare, Sydney, Australia) density gradient method [24]. Nuclear proteins were extracted and quantified then HDAC and HAT enzyme activity were measured (see Appendix for detailed method).

SCFAs (acetate, propionate and butyrate) were measured in plasma by gas chromatography (GC- Zebron FFAP 30 m × 530 μm × 1·0 μm)). Faecal SCFA concentrations were determined by flame ionisation detection using an Agilent Technologies 7890A gas chromatograph fitted with a flame-ionisation detector with nitrogen as the carrier gas [25]. Extraction and quantification of bacterial DNA from faecal samples, and sequencing of the V4 hypervariable region of the bacterial 16S rRNA gene, was performed as described previously. Quantitative PCR assays were used to assess levels of bacterial species as described previously [26]. Levels were normalised to total bacterial load, and differences resulting from treatment expressed as fold change [26].

2.6. Statistical analysis

Data were analysed using STATA 15 (StataCorp, College Station, Texas, USA). Continuous data were tested for non-normality using the D'Agostino-Pearson omnibus test. Per protocol analysis of study outcomes was performed using linear mixed effects modelling (LMMs) fit by restricted maximum likelihood, to assess the difference in the change in outcomes from baseline, between treatments and placebo. Treatment

group (inulin, inulin+probiotic or placebo) and time (treated as categorical with levels at pre and post-treatment) were specified as fixed effects with an interaction term. Measurements were repeated on the subject variable. LMMs use all data available at each time point; therefore missing data imputation was not undertaken. The difference in variable change between intervention arms and placebo is presented as coefficient [95% CI]. Exploratory within group comparisons of outcomes compared to baseline were conducted using Wilcoxon signed-rank tests. Exploratory subgroup analysis by inflammatory phenotype was also conducted using Wilcoxon rank-sum test. For microbiota data changes between groups were assessed using Kruskal-Wallis testing with Dunn's post hoc test. Associations were assessed using Spearman's rank correlation coefficient. Significance was accepted if $p < 0{\cdot}05$.

3. Results

From May 2015 to March 2016, 88 individuals were assessed for eligibility, 17 subjects ($n = 9$ females, $n = 8$ males) were randomised, and 3 subjects withdrew during the study (Fig. 2). Of subjects who completed the trial, adherence to the study supplements was 100% and adherence to the background diet (consumption of no more than two serves of fruit and vegetables during all treatment arms) was 88%. Gastrointestinal symptoms experienced during each treatment arm are reported in Table 1a. Following inulin treatment, subjects reported an increase in discomfort from indigestion, compared to baseline. Following inulin+probiotic, subjects reported an increase in diarrhoea compared to baseline. Adverse events are described in Table 1b. No

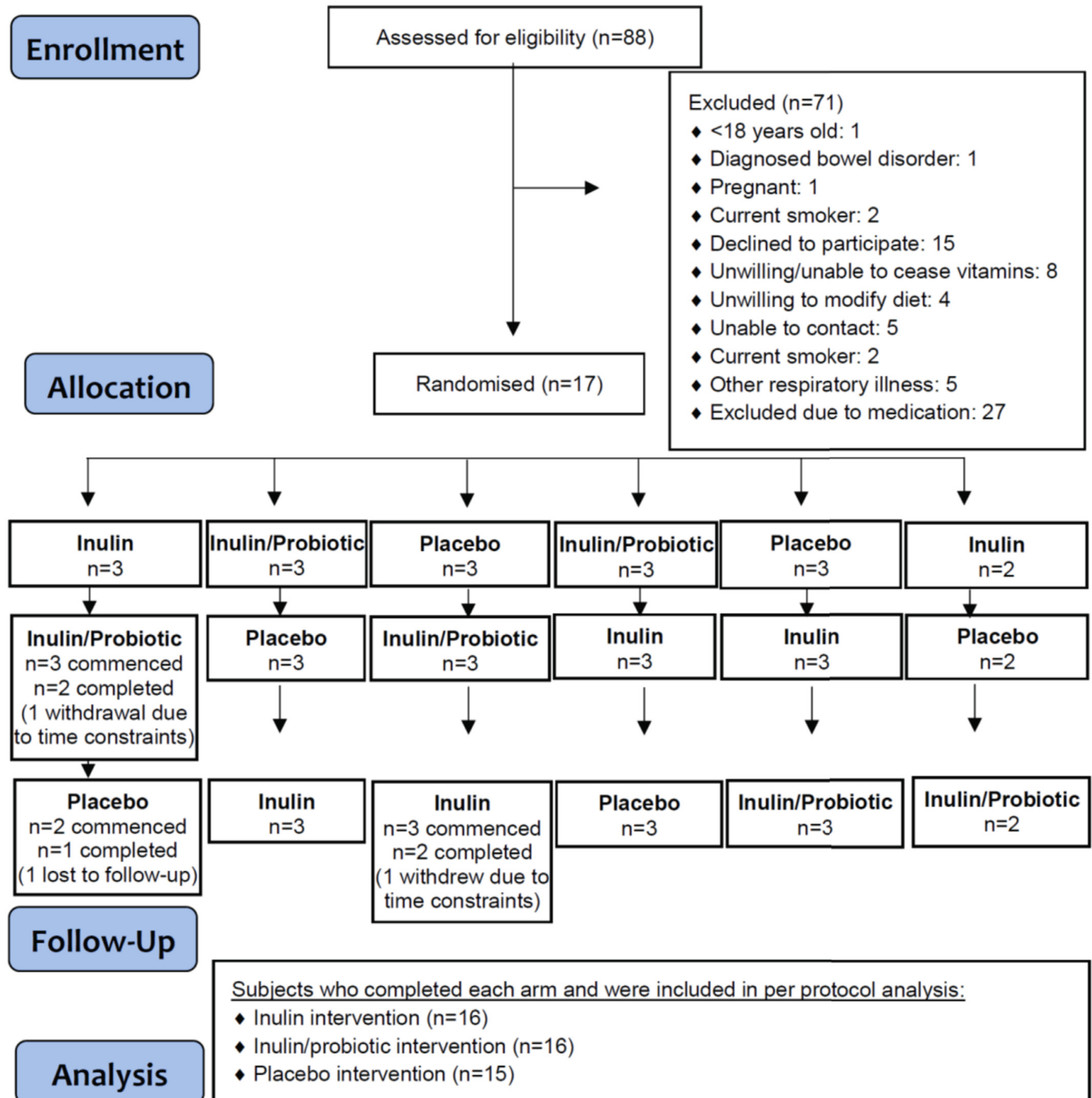

Fig. 2. CONSORT flow diagram.

Table 1a
Gastrointestinal symptoms rating scale (GSRS) domain scores.

Outcome	Pre[a]	Post[a]	Difference[d]
Abdominal			
Inulin	1.17 (1, 2)	1.33 (1, 2.17)	−0.27 [−0.80, 0.26]
Inulin + probiotic	1.33 (1, 2)[b]	1.33 (1, 1.5)	−0.52 [−1.05, 0.01]
Placebo	1 (1, 1.33)	1.33 (1, 1.67)[c]	
Reflux			
Inulin	1 (1, 1.5)	1 (1, 1)	−0.09 [−0.56, 0.37]
Inulin + probiotic	1 (1, 1)	1 (1, 1)	−0.04 [−0.50, 0.42]
Placebo	1 (1, 1.5)	1 (1, 1)	
Diarrhoea			
Inulin	1 (1,1.3)	1 (1,1.3)	−0.13 [−0.84, 0.58]
Inulin + probiotic	1 (1,1)	1.3 (1,1.7)[c]	−0.05 [−0.75, 0.65]
Placebo	1 (1,1.7)	1 (1,1.3)	
Indigestion			
Inulin	1 (1, 1.8)	1.3 (1, 2.5)[c]	0.39 [−0.08, 0.86]
Inulin + probiotic	1 (1, 1.5)	1.5 (1.3, 2)	0.33 [−0.15, 0.80]
Placebo	1 (1, 1.3)	1 (1, 1.5)	
Constipation			
Inulin	1.2 (1, 1.5)	1 (1, 1.7)	−0.13 [−0.49, 0.23]
Inulin + probiotic	1 (1, 1.3)	1 (1, 1.3)	−0.14 [−0.50, 0.21]
Placebo	1 (1, 1.3)	1 (1, 1.7)	

[a] Data are non-normally distributed and presented as median (IQR). GSRS scoring: 1 = no discomfort, 2 = slight discomfort, 3 = mild discomfort, 4 = moderate discomfort.
[b] Difference ($p > .05$) in pre intervention values between intervention arm and placebo analysed by mixed model regression accounting for repeated measures.
[c] Change within intervention arm analysed by Wilcoxon signed rank.
[d] Coefficient [95% CI] of difference in variable change between intervention arm and placebo analysed by linear mixed effects model accounting for repeated measures, GI, gastrointestinal; URTI, upper respiratory tract infection.

Table 2
Baseline demographics and clinical characteristics.

N	17
Age years (range)	43 (19–82)
Weight kg	87.2 ± 19.6
BMI kg/m^2 [a]	30.4 ± 5.7
Male n (%)	8 (47)
Female n (%)	9 (53)
Ex-smokers n (%)	5 (29)
Smoking history (pack years)	1 (0, 15)
Asthma pattern (intermittent/mild/moderate/severe persistent)	1/11/4/1
FEV_1% predicted[a]	76.1 ± 17.8
FVC % predicted[a]	76.2 ± 12.7
#FEV_1/FVC %[a]	70 ± 12.2
PD15 (mL)[a,c] (n = 9)	4.3 ± 3.4
DRS (%fall FEV_1/ mL saline)[b]	1.8 (0.6, 5.6)
ACQ6[a]	0.9 ± 0.5
SABA n (%)	16 (94)
Maintenance ICS n (%)	15 (88)
Maintenance ICS (μg beclomethasone equivalents/day)[b]	500 (400, 1000)
Oral steroids in 12 months n (%)	2 (12)

BMI, body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; DRS, Dose response slope; ACQ6, Asthma Control Questionnaire 6 [18]; SABA, short acting β^2-agonist; ICS, inhaled corticosteroids.
[a] Data are normally distributed and presented as mean ± SEM.
[b] Data are non-normally distributed and presented as median (IQR).
[c] PD15, provocation dose resulting in 15% fall in baseline FEV_1.

subjects were withdrawn due to gastrointestinal symptoms or other adverse events.

Subjects had a median age of 43 (19–82(range)) years, and mean BMI of 30·4 ± 5·7 kg/m^2 (Table 2). Most (88%, $n = 15$) subjects were using inhaled corticosteroids (ICS) and 47% ($n = 8$) had an ACQ6 score ≥ 0·75, indicating partial-poor asthma control. Following the interventions, there was no difference between groups in the change in total plasma SCFA, individual plasma SCFA (acetate, propionate and butyrate) (Fig. 3a, Table 3), faecal SCFA (Fig. 3b), or faecal pH (data not shown). Following both inulin and inulin+probiotic treatments there was a consistent pattern of increased total and individual plasma and faecal SCFA levels compared to baseline, which did not reach significance.

Post intervention, the were no differences between groups in the change in asthma control or airway inflammation. Within group analysis showed that following inulin supplementation there was a significant improvement in asthma control as indicated by a decrease in ACQ6 score (Δ−0·35(−0·50,−0·13) (med(IQR) $p = 0·006$) (Fig. 4, Table 4). Furthermore, 100% of subjects with partial-poorly controlled asthma (ACQ6 ≥ 0·75) prior to inulin treatment ($n = 8$) had a subsequent improvement in asthma control, with improvements reaching the minimum clinically important difference (MCID, 0.5) in 63% ($n = 5$) of these subjects [27]. There were no significant changes in lung function following any of the treatment arms (Table 4). However, following inulin treatment, change in FEV_1 was positively correlated with change in total faecal SCFAs ($R_s = 0·54$, $p = 0·045$), faecal butyrate ($R_s = 0·66$, p = 0·010) and faecal propionate ($R_s = 0·64$, p = 0·014) (Fig. 5). Sputum %eosinophils significantly decreased (Δ-1·0 (−2·5, 0·0) (med(IQR) p = 0·006) following inulin supplementation (Fig. 4, Table 4). No changes were observed in induced sputum total cell count (TCC), percentage or absolute cell count of airway neutrophils, macrophages and lymphocytes, following any of the treatment arms. (Table 4). Median FeNO concentrations before and after each treatment arm were in the normal range [28]. There was a small increase in FeNO following the placebo arm (Δ2·3 (0·7, 4·3)(med(IQR) $p = 0·046$), which reached statistical significance, yet is unlikely to be clinically significant [28].

There were no differences in gene expression of GPR41/43 following any of the treatments, or between the groups, in induced sputum (Appendix - Table S1) or PBMCs (Appendix -Table S2). However,

Table 1b
Number of subjects reporting mild, moderate or severe adverse events during the intervention and wash out periods, by study phase.

Phase	Event	Inulin			Inulin + probiotic			Placebo			Wash out		
		Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Phase 1	GI Symptoms									1			
	URTI	1											
	Haematoma				1								
Washout 1	URTI										1	3	
	Hayfever										2		
Phase 2	GI Symptoms	1						1					
	URTI				1								
	Headache		1				1						
	Tooth infection					1							
Washout 2	Migraine												1
	Headache										1		
	URTI											1	
	GI Symptoms												1
Phase 3	GI Symptoms		1						2				
	URTI							1					
	Arthritic pain		1										

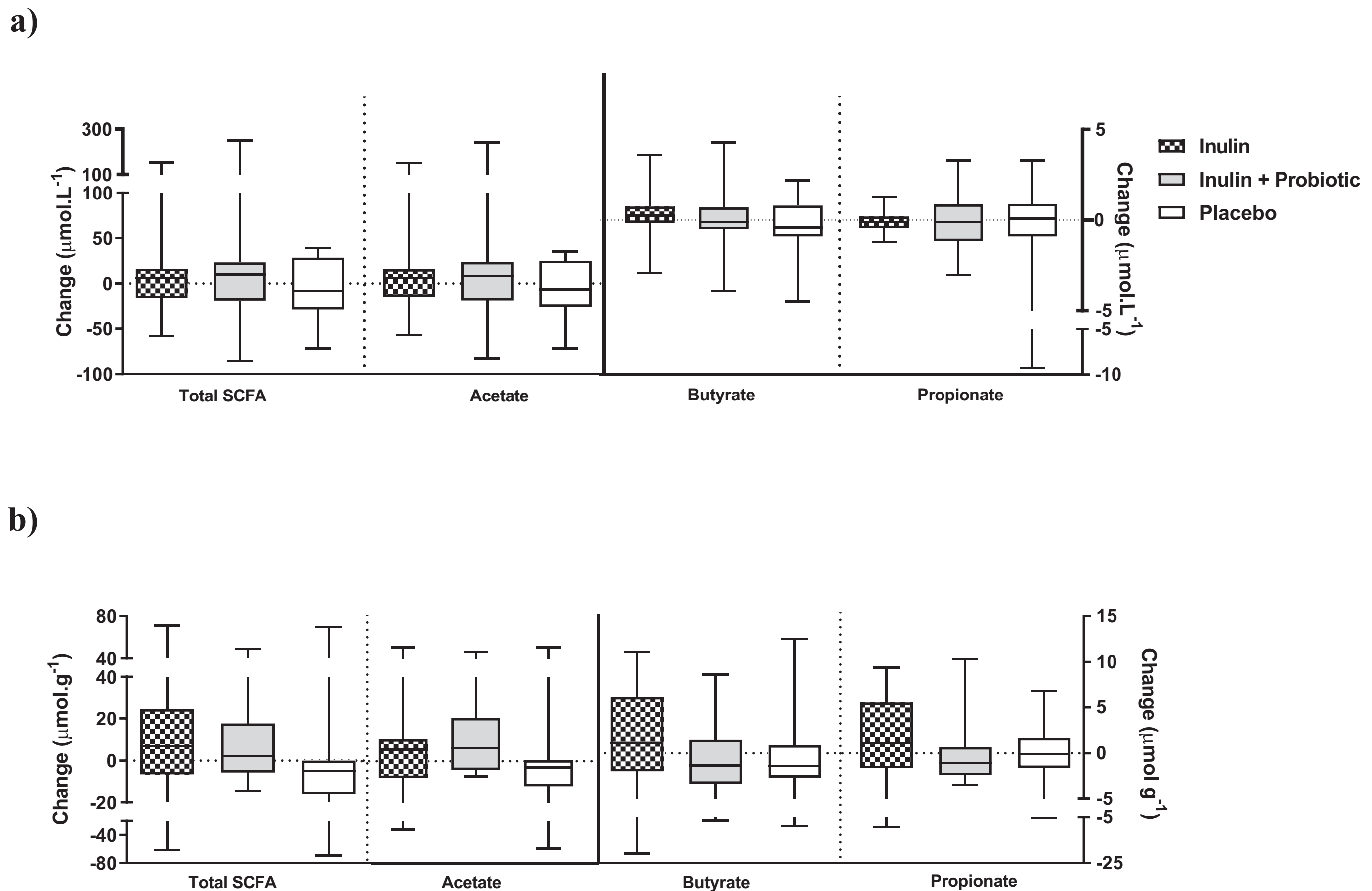

Fig. 3. Change in total SCFA, acetate, propionate and butyrate in a) plasma and b) faeces, following inulin, inulin+probiotic and placebo treatments. No changes within treatment arm analysed by Wilcoxon signed rank. No differences between treatment arms and placebo analysed by linear mixed effects model accounting for repeated measures. Data are reported as box and whisker plots, where the box represents median (IQR) and the whiskers represent range.

following inulin treatment there was a positive correlation between plasma butyrate and both GPR41 and GPR43 gene expression in PBMCs (Fig. 6). Total HDAC and HAT enzyme activity, total HAT/HDAC ratio and gene expression of HDAC subtypes in PBMCs did not significantly change following any of the treatment arms and there were no differences between the groups (Table 5). In induced sputum, gene expression of HDAC9 decreased (Δ-0·49 ($-0{\cdot}83,-0{\cdot}27$)(med (IQR), $p = 0{\cdot}008$) following treatment with inulin (Fig. 7)(Appendix -

Table 3
Plasma short chain fatty acids.

Outcome (μmol/L)	Pre[a,b]	Post[a]	Difference[c]
Total SCFA			
Inulin	112.7 (103.0, 162.9)	118.4 (84.7, 182.7)	16.3 [−16.9, 49.5]
Inulin + probiotic	128.6 (86.5, 157.6)	125.0 (93.5, 186.1)	18.7 [−14.5, 51.9]
Placebo	138.9 (79.9, 209.7)	126.4 (97.3, 177.2)	
Acetate			
Inulin	104.3 (92.3, 152.1)	109.8 (77.9, 172.1)	15.4 [−16.7, 47.6]
Inulin + probiotic	120.5 (78.3, 147.2)	114.9 (86.7, 173.3)	18.1 [−14.1, 50.2]
Placebo	131.1 (74.5, 195.8)	119.2 (88.9, 163.3)	
Butyrate			
Inulin	1.8 (1.4, 2.3)	1.9 (1.6, 3.2)	0.7 [−0.5, 1.9]
Inulin + probiotic	1.7 (1.2, 2.9)	2.2 (1.3, 3.1)	0.4 [−0.8, 1.6]
Placebo	2.2 (1.3, 2.6)	1.9 (1.6, 3.4)	
Propionate			
Inulin	6.0 (4.6, 9.1)	6.1 (4.5, 9.0)	0.2 [−1.1, 1.5]
Inulin + probiotic	6.8 (4.5, 9.7)	6.3 (5.1, 10.0)	0.2 [−1.1, 1.5]
Placebo	6.7 (4.8, 11.4)	5.8 (4.6, 11.0)	

SCFA, short chain fatty acids.
[a] Data are non-normally distributed and presented as median (IQR).
[b] No difference (p > .05) in pre-intervention values between intervention arms and placebo for all variables analysed by linear mixed effects model accounting for repeated measures; No changes within intervention arm analysed by Wilcoxon signed rank.
[c] Coefficient [95% CI] of difference in variable change between intervention arm and placebo analysed by linear mixed effects model accounting for repeated measures.

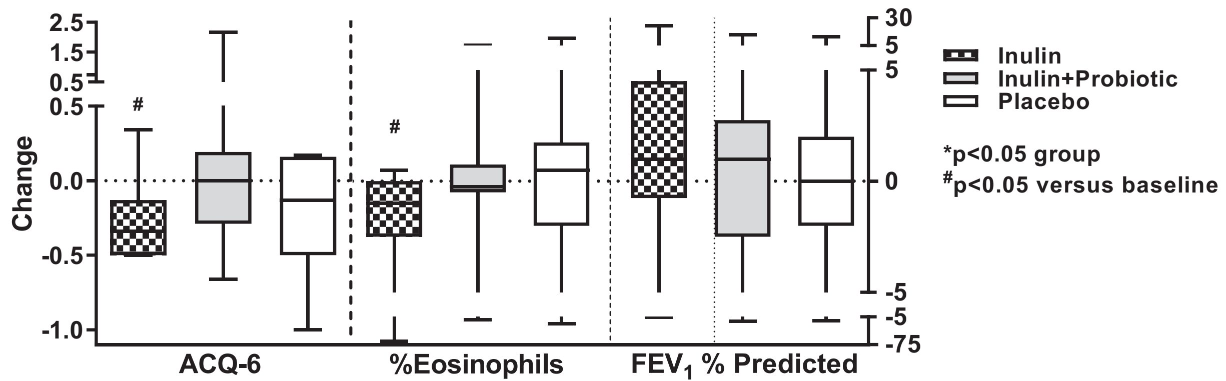

Fig. 4. Change in Asthma Control Questionnaire (ACQ) score, sputum %eosinophils and FEV_1%predicted following inulin, inulin+probiotic and placebo treatments. #Changes within treatment arm analysed by Wilcoxon signed rank. No differences between treatment arms and placebo analysed by linear mixed effects model accounting for repeated measures. Data are reported as box and whisker plots, where the box represents median (IQR) and the whiskers represent range.

Table S1). Furthermore, sputum %eosinophils were positively correlated with sputum HDAC9 gene expression ($R_s = 0{\cdot}88$, $p = 0{\cdot}0003$) (Fig. 8a).

Table 4
Lung function, asthma control score and airway inflammatory markers.

Outcome	Pre[a]	Post[a]	Difference[d]
FEV_1% predicted			
Inulin	74 (65, 83)	77 (71, 87)	3.2 [−1.5, 7.9]
Inulin + probiotic	74 (66, 84)[b]	80 (67, 88)	0.4 [−4.3, 5.2]
Placebo	80 (64, 93)	80 (71, 92)	
FVC % predicted			
Inulin	87 (79, 92)	91 (81, 95)	2.2 [−2.4, 6.7]
Inulin + probiotic	87 (78, 92)	87 (78, 94)	−2.4 [−7.0, 2.1]
Placebo	91 (77, 102)	89 (83, 93)	
FEV_1/FVC %			
Inulin	67 (58, 80)	73 (59, 80)	1.3 [−1.4, 3.9]
Inulin + probiotic	73 (60, 80)[b]	74 (58, 80)	1.3 [−1.3, 3.9]
Placebo	72 (57, 80)	76 (56, 80)	
ACQ6			
Inulin	0.8 (0.5, 1.2)	0.3 (0.3, 0.8)[c]	−0.04 [−0.39, 0.32]
Inulin + probiotic	0.8 (0.5, 0.8)	0.7 (0.3, 1.3)	0.31 [−0.04, 0.67]
Placebo	1.0 (0.5, 1.3)	0.5 (0.3, 1.2)	
Total cell count			
Inulin	4.1 (2.4, 8.8)	5.7 (3.3, 9.3)	0.6 [−3.2, 4.4]
Inulin + probiotic	6.0 (2.5, 7.3)	5.7 (3.0, 9.5)	−1.1 [−4.9, 2.6]
Placebo	5.5 (3.2, 9.5)	5.4 (3.3, 8.3)	
Eosinophils %			
Inulin	2.3 (1.3, 5.1)	1.5 (0.5, 2.8)[c]	−3.8 [−11.1, 3.5]
Inulin + probiotic	1.0 (1, 4.5)	1.3 (0.5, 3.8)	0.29 [−7.0, 7.6]
Placebo	1.3 (0.3, 6)	2.0 (0.8, 5)	
Neutrophils %			
Inulin	52.7 (21.8, 67.4)	46.8 (26.0, 71.3)	1.3 [−12.2, 14.7]
Inulin + probiotic	49.0 (19.8, 59.0)	31.3 (17.3, 62.0)	0.4 [−13.0, 13.8]
Placebo	52.3 (13.8, 61.8)	47.5 (25.0, 63.3)	
Macrophages %			
Inulin	40.0 (20.1, 61.5)	48.0 (24.0, 69.5)	2.0 [−12.3, 16.4]
Inulin + probiotic	41.5 (33.3, 72.5)	54.0 (31.8, 75.3)	−3.0 [−17.3, 11.2]
Placebo	42.8 (28.5, 66.5)	44.8 (31.5, 67.0)	
Lymphocytes %			
Inulin	1.6 (0.8, 2.5)	1.8 (1.0, 2.5)	−0.8 [−2.8, 1.3]
Inulin + probiotic	1.5 (0.8, 4.0)[b]	1.8 (0.3, 3.0)	−1.0 [−3.0, 0.9]
Placebo	0.8 (0.3, 1.3)[b]	1.5 (0.3, 3.8)	
FeNO ppb			
Inulin	11.0 (5.5, 18.0)	11.8 (6.2, 16.9)	−1.8 [−7.1, 3.4]
Inulin + probiotic	11.0 (6.8, 18.2)	13.6 (6.1, 17.3)	−1.9 [−7.1, 3.2]
Placebo	11.2 (6.1, 16.4)	8.4 (7.0, 19.6)[c]	

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; ACQ6, Asthma Control Questionnaire 6; FeNO, fraction of exhaled nitric oxide.
[a] Data are non-normally distributed and presented as median (IQR).
[b] Significantly different ($p < .05$) pre-intervention value compared to placebo group analysed by linear mixed effects model accounting for repeated measures.
[c] Change within treatment arm analysed by Wilcoxon signed rank.
[d] Coefficient [95% CI] of difference in variable change between intervention arm and placebo analysed by linear mixed effects model accounting for repeated measures.

Subjects were classified as eosinophilic asthma or non-eosinophilic asthma, using induced sputum cell counts. Prior to inulin treatment eosinophilic asthmatics had worse asthma control ($p = .033$) and higher sputum cell HDAC9 expression ($p = .033$) than non-eosinophilic asthmatics (Fig. 8b). In eosinophilic asthmatics following inulin supplementation, clinically important improvements were observed in asthma control ($n = 7$, Δ-0·5(−0·5, −0·13)(med(IQR) $p = 0{\cdot}018$) and sputum cell HDAC9 expression significantly decreased ($p = 0{\cdot}043$) (Fig. 8c). Gene expression of all other HDACs (HDAC1–8, 10 & 11) did not differ according to asthma inflammatory phenotype.

In keeping with previous studies of faecal microbiology, we observed significant differences in microbiota composition between individuals ($p = 0{\cdot}0001$, pseudo-$F = 8{\cdot}62$, 9721 permutations). However, microbiota composition did not cluster significantly with treatment ($p = 0{\cdot}147$, pseudo-$F = 1{\cdot}11$, 9708 permutations) (Appendix – Fig. S1a). Within subject assessment of pre/post microbiota similarity also showed no difference between treatment groups (Bray Curtis similarity: $H = 1{\cdot}626$, $p = 0{\cdot}4435$) (Appendix – Fig. S1b). Measures of bacterial alpha diversity, reflecting a number of different aspects of microbiota structure, also showed no significant difference between treatment groups ($p > 0{\cdot}6$). Despite the absence of an effect on overall microbiota composition, significant changes in the levels of individual bacterial operational taxonomic units (OTUs) were observed (assessed as median fold change compared to baseline) following both inulin and inulin+probiotic arms. The relative abundance of OTUs corresponding to *Bifidobacterium* increased significantly in response to treatment with inulin+probiotic which was significantly different to placebo (Fig. 9) and showed a non-significant trend towards increase in response to treatment with inulin alone ($p = 0{\cdot}058$). The relative abundance of OTUs corresponding to *Anaerostipes* increased significantly in response to treatment with both inulin and inulin+probiotic; the response to inulin was significantly different to placebo (Fig. 9). The relative abundance of OTUs corresponding to *Roseburia* decreased after inulin treatment alone. A decrease in *Erysipelotrichaceae* was also observed after inulin treatment alone and this was significantly different to the change following placebo (Fig. 9). 16S rRNA gene amplicon sequencing provides only a limited basis for bacterial taxon identification and a relative, rather than an absolute, measure of taxon abundance. We therefore used a panel of species-specific qPCR assays to quantify the three principal *Bifidobacterium* species in adult humans (*B. adolescentis*, *B. longum*, and *B. catenulatum* group), the *Anaerostipes hadrus* group, the *Eubacterium rectale*/*Roseburia* group, and the *Faecalibacterium prausnitzii* group. A significant increase in *B. adolescentis* ($p = 0{\cdot}010$) and *B. longum* ($p = 0{\cdot}040$) abundance was observed following inulin+probiotic treatment compared to placebo (Fig. S2).

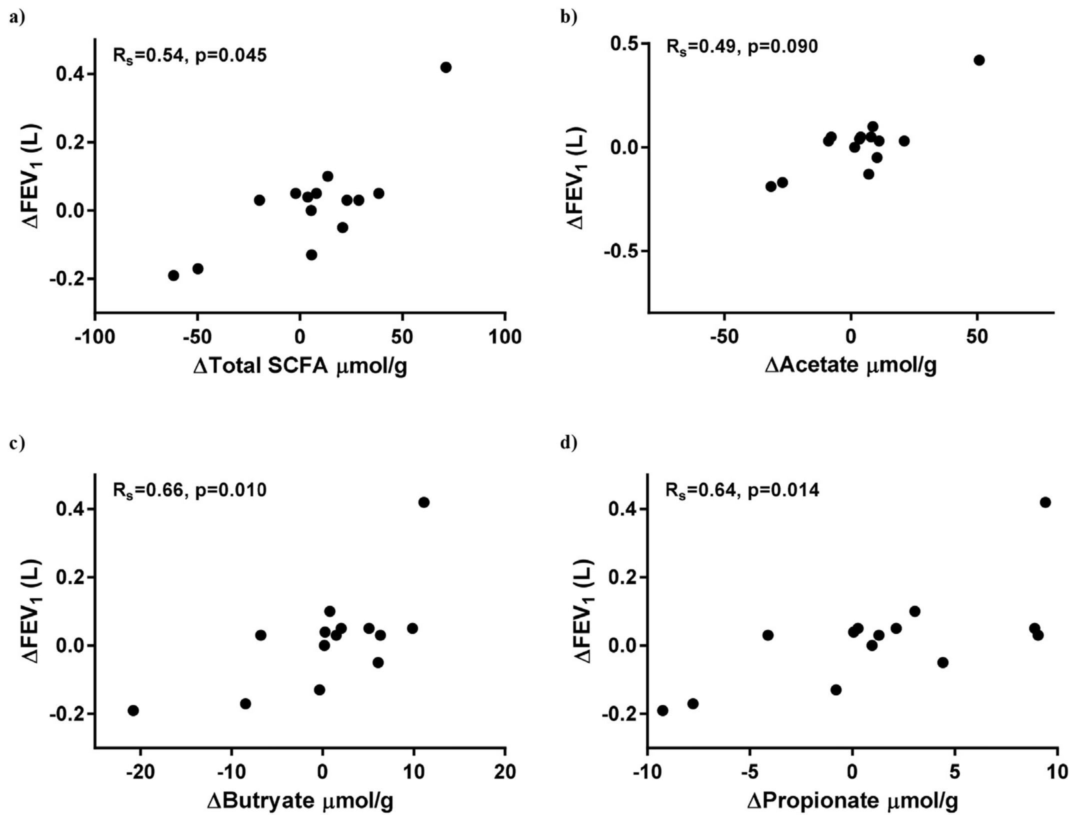

Fig. 5. Following inulin treatment, the association between change (Δ) in lung function (FEV_1) and Δfaecal SCFA concentrations including a) ΔTotal SCFA, b) ΔAcetate, c) ΔButyrate and d) ΔPropionate. Data analysed using Spearman's rank correlation coefficient (R_s).

In the inulin treatment group, we observed inverse correlations between the relative abundance of *Bifidobacterium* at visit 2 and the change in airway responsiveness (measured as DRS) ($R_s = -0.5857$, $p = .0218$) and the relative abundance of *Anaerostipes* at visit 2 and the change in total sputum cell count ($R_s = -0.5604$, $p = .0463$). We also observed positive correlations between the change in relative abundance of *Roseburia* and the change in DRS ($R_s = 0.6352$, $p = .0147$) and the relative abundance of *Roseburia* at visit 2 and the change in DRS ($R_s = 0.6250$, $p = .0127$).

4. Discussion

This study aimed to investigate the effect of supplementation with the soluble fibre inulin, with and without a probiotic, on plasma SCFA, clinical outcomes, airway inflammation and gut microbiota in asthma. In addition, potential anti-inflammatory mechanisms of soluble fibre in asthma were examined. There were no changes in the primary outcome, plasma SCFAs, following any of the interventions, however, posthoc analysis identified several changes following 7 days of inulin supplementation only, that warrant further investigation. Eosinophilic airway inflammation decreased and asthma control also significantly improved with inulin supplementation only, with the greatest improvements observed in subjects who had poorly controlled asthma at baseline, despite being on inhaled corticosteroids. No significant changes in clinical outcomes or airway inflammation were observed following inulin+probiotic or placebo treatment. We also found a significant decrease in HDAC9 gene expression in sputum cells following inulin supplementation, and HDAC9 expression was associated with %sputum

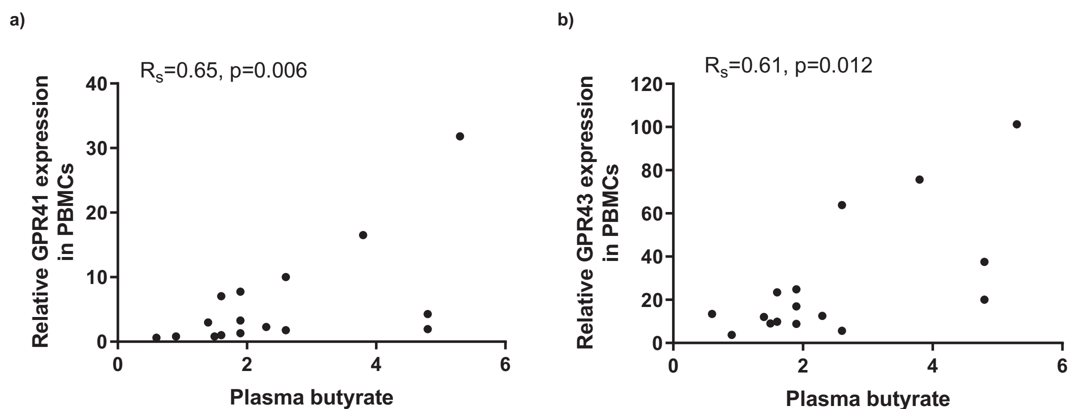

Fig. 6. Following inulin treatment, the association between plasma butyrate concentrations and PBMC expression of a) GPR41 and b) GPR43. Data analysed using Spearman's rank correlation coefficient (R_s). Gene expression data are reported as $2^{-\Delta Ct}$ (x10 [6]) relative to the housekeeping gene18S.

Table 5
HDAC and HAT activity in peripheral blood mononuclear cells.

	N	Pre[a,b]	Post[a]	Difference[c]
HDAC activity (μM/μg)				
Inulin	16	3.43 (3.03, 4.28)	3.82 (3.47, 4.58)	0.31 [−0.52, 1.13]
Inulin + probiotic	16	4.35 (3.54, 4.72)	3.78 (3.24, 4.54)	−0.53 [−1.36, 0.30]
Placebo	15	3.63 (3.03, 4.35)	3.90 (3.70, 4.46)	
HAT activity (μM/μg)				
Inulin	14	5.65 (3.01, 10.39)	4.26 (2.47, 10.06)	0.16 [−4.60, 4.91]
Inulin + probiotic	16	4.93 (1.88, 9.57)	4.33 (1.83, 8.99)	−0.32, [−4.96, 4.32]
Placebo	14	5.51 (2.86, 9.25)	5.65 (2.59, 7.41)	
HAT:HDAC ratio				
Inulin	14	1.55 (0.64, 2.82)	0.97 (0.60, 2.97)	0.26 [−1.34, 1.87]
Inulin + probiotic	16	1.12 (0.47, 2.67)	1.06 (0.40, 2.19)	0.10 [−1.46, 1.67]
Placebo	14	1.11 (0.75, 3.07)	1.25 (0.66, 1.85)	

HDAC, histone deacetylase; HAT, histone acetyltransferase.
[a] Data are non-normally distributed and presented as median (IQR).
[b] No difference ($p > .05$) in pre-intervention values between intervention arms and placebo for all variables analysed by linear mixed effects model accounting for repeated measures; No changes within treatment arm analysed by Wilcoxon signed rank.
[c] Coefficient [95% CI] of difference in variable change between intervention arm and placebo analysed by linear mixed effects model accounting for repeated measures.

eosinophils. Importantly, all supplements were well tolerated, with only small increased in gastrointestinal symptoms being observed.

This study provides preliminary evidence of the anti-inflammatory effects of soluble fibre in adults with asthma, as within group analysis revealed that inulin supplementation decreased airway eosinophils, a finding supported by previous studies conducted in both animals and humans. In one study, mice fed a diet high in soluble fibre (pectin) were protected against allergic inflammation in the lungs and showed improved airway hyper responsiveness [4]. In another study using a house-dust mite model of allergic airways disease (AAD), a high-fibre diet (high amylose maize resistant starch) protected against the development of AAD [29]. In an observational study, we have previously shown that dietary fibre intake is inversely associated with eosinophilic airway inflammation and positively associated with lung function in adults with asthma [5]. Further, in an acute meal challenge model, we demonstrated that a single dose of soluble fibre (inulin) was effective in reducing airway inflammation in adults with asthma [6].

In our intervention, within group analysis revealed that inulin supplementation significantly improved asthma control, with the greatest improvements observed in those whose asthma was not well-controlled prior to supplementation, despite using inhaled corticosteroids. Of these individuals, 63% had a clinically important improvement in asthma control, which is defined as a change in ACQ6 ≥ 0.5 [18]. Interestingly, our results also demonstrate that eosinophilic asthmatics had worse asthma control than non-eosinophilic asthmatics prior to inulin supplementation, with significant and clinically important improvements in asthma control following inulin supplementation observed in individuals with eosinophilic asthma, but not in those with non-eosinophilic asthma. Our results agree with previous studies showing that subjects with poorly controlled asthma have higher levels of airway eosinophils [30,31] and suggests that inulin supplementation warrants further investigation as a suitable therapy for such patients.

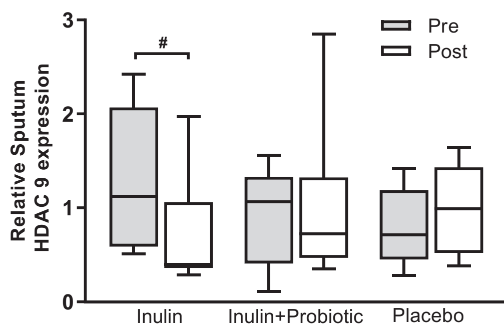

Fig. 7. Change in sputum cell HDAC9 gene expression following inulin, inulin+probiotic and placebo treatments. Gene expression data are reported as $2^{-\Delta Ct}$ (x10 [6]) relative to the housekeeping gene 18S. [#]Changes within treatment arm analysed by Wilcoxon signed rank. No differences between treatment arms and placebo analysed by linear mixed effects model accounting for repeated measures. Data are reported as box and whisker plots, where the box represents median (IQR) and the whiskers represent range.

Soluble fibres, such as inulin, are partially fermented by beneficial bacteria in the colon, which increases their number and diversity, and generates physiologically active by-products including the SCFAs: acetate, propionate, and butyrate, which have anti-inflammatory properties [4]. Butyrate is primarily absorbed by colonocytes, being their major energy source; propionate is mostly absorbed by the liver; and acetate is the primary SCFA that enters the circulation, which may be most relevant to diseases of the peripheral organs, such as asthma. Despite shifts in microbiota composition and patient-reported increase in discomfort from indigestion that indicate colonic fermentation of inulin, we observed only small increases in plasma SCFA following the inulin and inulin+probiotic treatments, which did not reach statistical significance. We speculate that the lack of significance of these changes was due to the timing of venous blood sampling, which occurred after a 12 h fast. Following a single soluble fibre dose, plasma SCFA levels peak at 4–6 h, returning to baseline at 8–10 h [32]. Hence the sample was not taken during the postprandial SCFA peak. In future studies, it is recommended to measure plasma SCFAs at 4–6 h post-supplementation to accurately determine changes in SCFA production.

Similarly, we observed only small increases in faecal SCFA levels, which were not statistically significant. This is consistent with previous studies [26,33,34] and may reflect the efficient uptake of the majority of SCFA produced by the colonic epithelium [34], with relatively low faecal excretion. Nonetheless, following inulin supplementation, changes in FEV_1 were positively correlated with changes in total faecal SCFA, faecal butyrate and faecal propionate. In keeping with previous analyses of the faecal microbiota composition in adults [35], we observed significant inter-individual variation, including baseline levels of bifidobacteria. These differences are likely to contribute to variation in response to inulin supplementation as observed here, and as reported elsewhere [36–39]. We were unable to assess whether SCFAs reached the lungs in our study. One previous animal study was unable to detect SCFAs in lung tissue following soluble fibre supplementation, despite increases in serum and cecum SCFA levels [4]. It is however suggested that irrespective of whether SCFA enter the airways, the immunomodulatory effects of SCFAs in the airways initially occur systemically [4].

Interactive communities of anaerobic microbes in the colon are required to ferment non-digestable carbohydrates, such as inulin, to produce SCFAs. Bifidobacteria are particularly important in the fermentation of inulin-type fructans (ITFs) [36,40], although other saccharolytic members of the colon microbiota, including *Lactobacillus* spp., *Bacteroides* spp., *Roseburia* spp., *Eubacterium rectale* and *F. prausnitzii*, can also contribute to this process [41–43]. Competition to utilise inulin, and cross-utilisation of the resulting metabolites, influences colonic microbiota composition [44–46], and determines the release of SCFAs into the gut lumen [45]. In keeping with previous research [26], inulin did not change the relative abundance of the majority of bacterial groups, likely reflecting the relatively low abundance of the principal utilisers of ITFs within the wider colonic bacterial community [47]. However, we did observe a significant increase in the abundance of bifidobacteria, both in terms of relative abundance and absolute numbers. Bifidobacteria are able to degrade inulin to produce acetate and lactate [48]. Previous studies have reported increases in bifidobacteria in response to inulin supplementation [26,44,49], particularly, as here, in *B. adolescentis* [26]. Notably, the observed increase in *B. adolescentis* only achieved statistical significance in subjects who

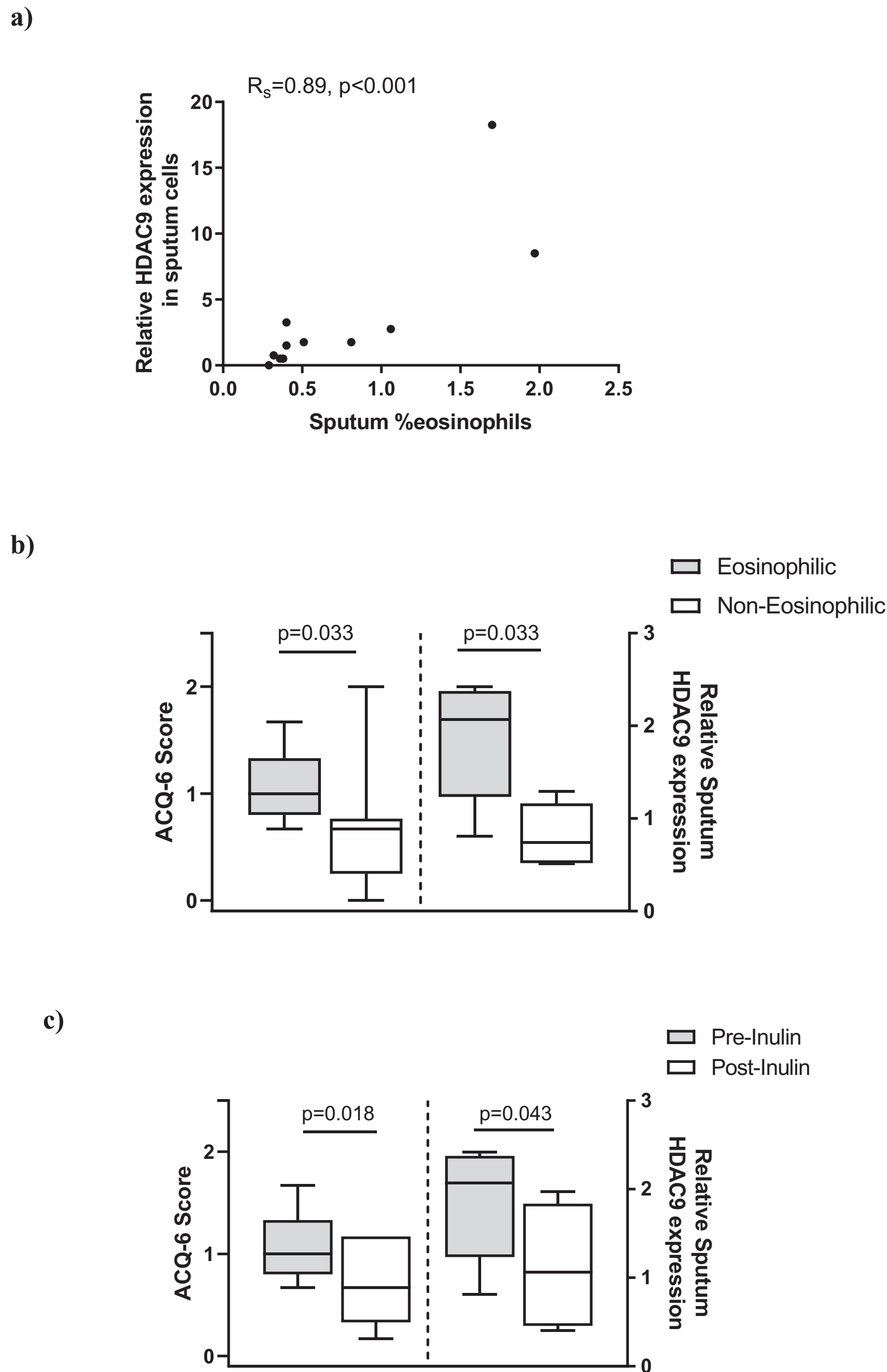

Fig. 8. a) Following inulin treatment, the association between sputum %eosinophils and sputum HDAC9 gene expression. Analysed using Spearman's rank correlation coefficient (R_s). b) Comparison of asthma control (ACQ6 score) and sputum cell HDAC9 gene expression in eosinophilic versus non-eosinophilic asthma prior to inulin supplementation. Data analysed using Wilcoxon rank-sum test. c) ACQ6 score and sputum cell HDAC9 gene expression in eosinophilic asthma, before and after inulin supplementation. Data analysed using Wilcoxon signed rank test. Gene expression data are reported as $2^{-\Delta Ct}$ (×10 [6]) relative to the housekeeping gene18S. Data are reported as box and whisker plots, where the box represents median (IQR) and the whiskers represent range.

received inulin in combination with the probiotic. However, the bifidobacterial strain included in the probiotic, *Bifidobacterium animalis* subspecies *lactis* BB-12, is phylogenetically distinct from *B. adolescentis* [50] and is therefore not directly responsible for the increase in the bifidobacterial population [50]. Similarly, despite the inclusion of two strains of *Lactobacillus* in the administered probiotic (*L. acidophilus* LA-5 and L. *rhamnosus* GG), no changes in *Lactobacillus* relative abundance were observed following combined supplementation with inulin and probiotic, which again suggests that the probiotic used has not directly influence microbiota changes.

B. adolescentis is one of the two dominant bifidobacterial species found in the adult colon, along with *B. longum* [51]. These species contain high numbers of genes involved in the degradation of carbohydrates of food origin [52,53] and displace *B. bifidum* and *B. longum*, which are adapted to metabolising human milk oligosaccharides [51,54] and dominate during infancy [51]. While bifidobacteria compete

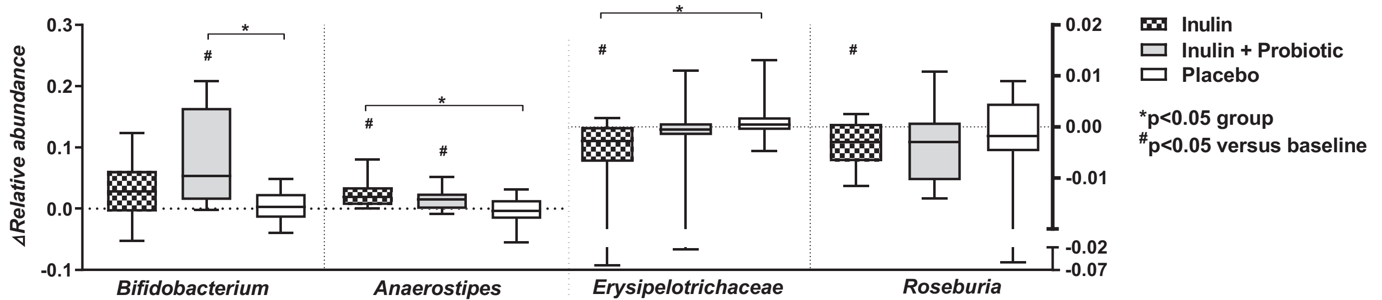

Fig. 9. Change in relative abundance of microbiota species following inulin, inulin+probiotic and placebo treatments. Gene expression data are reported as fold change. #Changes within treatment arm compared to baseline were assessed using Wilcoxon signed rank test. *Differences between treatment arms and placebo assessed using Kruskal-Wallis testing with Dunn's post hoc test. Data are reported as box and whisker plots, where the box represents median (IQR) and the whiskers represent range.

with other members of the commensal microbiota to utilise inulin, their production of acetate and lactate forms the basis for cross-feeding interactions with other species, including *Anaerostipes* spp., *Eubacterium* spp., and *Roseburia* spp., belonging to *Clostridium* cluster XIVa, and *Butyricicoccus pullicaecorum* and *Faecalibacterium prausnitzii*, belonging to Clostridium cluster IV [45,55–60]. Hence, the changes that we observed are likely to be interdependent. We observed an increase in the relative abundance of *Anaerostipes* and a decrease in the relative abundance of *Roseburia* following inulin supplementation. This may reflect a competitive relationship between these taxa, as they compete for niche space, leading to reciprocal shifts in relative abundance that reflect competitive exclusion. Interestingly, following inulin supplementation, the decrease in *Roseburia* and the higher relative abundance of *Bifidobacterium* correlated with an improvement in airway hyperresponsiveness. In addition, the higher relative abundance of *Anaerostipes* following inulin supplementation correlated with a decrease in sputum inflammatory cell count. These observations suggest that inulin induced changes in these taxa that have a positive influence in the airways, which may be related to changes in SCFA production.

SCFAs are suggested to have anti-inflammatory effects via activation of GPR41/43 on immune cells [14,61]. Several animal models of allergic airways disease have demonstrated that GPR41/43 activity is necessary for the resolution of airway inflammation [4,62]. In humans, we have also previously observed an increase in GPR41 and GPR43 expression in airway immune cells in adults with asthma following a single dose of soluble fibre, which corresponded with a decrease in airway inflammation [6]. However, in the current study, no significant changes in GPR41 or GPR43 were observed following inulin or inulin + probiotic supplementation compared to placebo. This suggests that the effects of soluble fibre may change over time. Nonetheless, our data show a positive correlation between plasma butyrate levels and GPR41/43 gene expression in PBMCs. Hence, further research is needed to explore this potential anti-inflammatory mechanism of soluble fibre in asthma.

SCFAs are also proposed to modulate inflammation by inhibiting the activity of HDAC enzymes, which together with HAT enzymes, are involved in the epigenetic regulation of gene expression [63]. Although we did not observe any significant changes in total HDAC/HAT enzyme activity, following 7 days of inulin supplementation within group analysis revealed a significant decrease in HDAC9 gene expression in sputum cells. Previous evidence demonstrates that HDAC9 gene expression is elevated in individuals with asthma compared to those without asthma [64]. Experiments using HDAC9 knockout mice, have suggested that HDAC9 deletion leads to increased regulatory T cells (Treg cells) [65], which are known to suppress the development of AAD [66]. Treg cells play an important role in asthma, with asthmatics having fewer and less functional Treg cells compared to their non-asthmatic counterparts [29], leading to enhanced Th2-type immune responses, including eosinophil influx into the airways [67]. Studies have shown that gene deletion or knockout of HDAC9 can increase the suppressive function of Treg cells [68–70]. Linking these observations with our intervention, a study conducted in mice by Thornburn et al. demonstrated that a high-fibre diet, via the production of acetate, primes Treg cell protection against asthma, likely through the inhibition of HDAC9 [29]. In the current study, prior to supplementation, eosinophilic asthmatics had significantly higher sputum HDAC9 expression than non-eosinophilic asthmatics, with a significant decrease in HDAC9 expression following inulin supplementation observed in eosinophilic asthmatics only. Furthermore, following inulin supplementation sputum HDAC9 gene expression was positively correlated with sputum %eosinophils. It is possible that down regulating HDAC9 gene expression suppressed eosinophilic influx into the airways via enhanced Treg cell function. Further research is warranted to explore HDAC9 as a potential therapeutic target for asthmatics, particularly those with eosinophilic airway inflammation.

Interestingly, our data do not support the use of combined inulin +probiotic supplementation, with no significant changes in clinical outcomes or airway inflammation observed. Evidence for the use of combined prebiotics and probiotics (i.e. synbiotics) is conflicting. In our recent systematic review, only 50% of studies examining the anti-inflammatory effects of synbiotics reported a significant decrease in systemic inflammation [2]. Furthermore, only 43% of included studies which compared prebiotics and synbiotics reported greater anti-inflammatory effects with synbiotic versus prebiotic supplementation [2]. While it is difficult to explain why inulin alone provided benefits that were not also seen with the symbiotic treatment, the differences may relate to the changes in bacterial OTUs that occurred in the inulin only, but not the inulin + probiotic, arms. The relative abundance of OTUs corresponding to *Anaerostipes*, a SCFA producing-bacteria [71], increased significantly in response to treatment with inulin but not inulin +probiotic. Conversely, after inulin alone but not inulin+probiotic, there was a decrease in the relative abundance of *Roseburia* and *Erysipelotrichaceae*, which has been shown to be highly immunogenic with a role in inflammation [72]. Furthermore changes in *Roseburia* and relative abundance of *Anaerostipes* were associated with improvements in airway hyperresponsiveness and airway inflammation respectively. The functional significance of these changes in bacterial OTUs, which likely alter SCFA production [41–43,71], warrants investigation in future studies.

A limitation of this study is the sub-optimal time point at which we measured plasma SCFAs (following a 12 h fast), which missed the post-prandial SCFA peak. This should be taken into consideration when designing future studies. Importantly, the improvements that we observed in airway inflammation, asthma control, gut microbiome and HDAC activity, were post hoc and preliminary, thus future appropriately powered trials are required to confirm these observations. Future work should also examine longer duration of treatment. The 7 day intervention and 14 day washout period were chosen based on the review by Roberfroid [73] which noted that interventions using inulin-type fructans lead to increases in bifidobacteria which become significant and maximal in less than a week, remain as long as the intake of the

prebiotic continues and progressively disappear when the intake stops (within 1–2 weeks) [73]. Asthma control can also change within a 7 day period and the ACQ6 is a suitable tool for identifying such short-term changes in asthma status, being validated to identify clinically meaningful changes in the previous week [74]. However, a longer study duration may be needed to see improvements in other asthma outcomes, such as lung function. Monitoring of gastrointestinal symptoms over a longer period is also warranted. While the study supplements were well tolerated over the 7 day intervention period, the effects of soluble fibre on gastrointestinal symptoms are likely to change over time. Interestingly, by chance, the mean BMI of our cohort was in the obese range. This is not dissimilar to the general population, in whom obesity prevalence is high and increasing (31% in Australia [75]; 40% in USA [76]). The modifying effect of obesity on responses to soluble fibre supplementation is unknown and is another interesting area for future research.

In summary, this study provides preliminary evidence for the benefits of soluble fibre in asthma, with improvements in asthma control and airway inflammation observed following 7 days of inulin supplementation. Our data suggest that individuals with poorly controlled asthma and eosinophilic airway inflammation, despite the use of inhaled corticosteroids, are most likely to benefit from inulin supplementation. Furthermore, HDAC9 inhibition has been identified as a likely mechanism of action. Being a non-pharmacological treatment, soluble fibre supplementation has the potential to be widely adopted and accepted as a strategy for managing asthma. Nonetheless, larger and longer duration clinical trials are needed to confirm these preliminary findings and to further explore HDAC9 as a potential therapeutic target for asthmatics, particularly those with eosinophilic airway inflammation.

Role of the funding source

The funding body had no role in study design, data collection, data analysis or interpretation or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Contributors

LGW, KJB, PGG and BSB designed and planned the study, which was supervised by LGW, KJB and PGG. BSB participated in recruitment and data collection. RM and EJW performed, analysed and interpreted laboratory analysis of sputum and blood samples. LEX and GBR performed, analysed and interpreted faecal microbiome analysis. RZ and BSB performed the statistical analysis and wrote the article, with input from all authors.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2019.07.048.

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